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INTERACTION OF NOVEL MONOMETHINE CYANINE DYES WITH PROTEINS IN NATIVE AND AMYLOID STATES[†]

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Molecular interactions between novel monomethine cyanine dyes and non-fibrillar and fibrillar proteins were characterized using the fluorescence spectroscopy and molecular docking techniques. To this end, the fluorescence spectral properties of the dyes have been explored in buffer solution and in the presence of insulin and lysozyme in the native and amyloid states. It was observed that association of monomethines with the native and fibrillar proteins was accompanied with a significant enhancement of the fluorophore fluorescence, being more pronounced in the presence of aggregated insulin and lysozyme. The quantitative information about the dye-protein binding was obtained through approximating the experimental dependencies of the fluorescence intensity increase *vs* protein concentration by the Langmuir model. Analysis of the spectral properties and the binding characteristics of monomethines in the presence of fibrillar insulin and lysozyme showed that the introduction of chloro- and fluorine-substituents to the oxazole yellow derivatives, as well as the long aliphatic substitution on the nitrogen atom of the benzazole chromophore of YO-dyes had a negative impact on the dye amyloid specificity. Molecular docking studies showed that monomethines tend to form the most stable complexes with the B-chain residues Val 17, Leu17, Ala 14, Phe1, Gln 4 and Leu 6 and the A-chain residue Leu 13, Tyr 14, Glu 17 of non-fibrillar insulin and interact with the deep cleft of native lysozyme lined with hydrophobic (Ile98, Ile 58), polar (Thr108, Thr 62, Thr 63) and negatively charged (Asp101, Asp 107) residues. The wet surface groove Gln15_Glu17 and groove G2-L4/S8-W10 were found as the most energetically favorable binding sites for the examined monomethine dyes in the presence of insulin and lysozyme fibrils, respectively.
Keywords: Monomethine cyanine dyes; insulin; lysozyme; amyloid fibrils; molecular docking.
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The monomethine cyanine dye oxazole yellow (YO) and its derivatives have continuously attracted tremendous interest in a variety of biomedical applications: i) as effective fluorescent probes for the DNA detection [1-5], sizing, purification of DNA fragments [6] and visualization of a single DNA molecules using fluorescent microscopy [7, 8]; ii) as selective indicators for the internal loop of the bacterial A-site RNA [9]; iii) for the production of the nanohybrid fluorescent materials based on the self-assembling the DNA wrapped carbon nanotubes and YO dyes [10]; iv) for monitoring the bacteriophage T5 capsid permeability for small molecules [11] and for detection and characterization of the MS2 bacteriophage [12]; v) for synthesis a dsDNA-sensitive fluorescent oxazole yellow-peptide bioconjugates [13]; vi) for detection of amyloid protein aggregates [14,15], to name only a few. These research activities are driven by the advantageous photophysical properties of YO derivatives, namely: i) high molar extinction coefficients; ii) high quantum yield and a strong fluorescent enhancement in the presence of specific biomolecules; iii) an ability to switch from a "dark state" to a fluorescent state in the presence of a restrictive environment such as base-pairs of double stranded DNA or amyloid fibril beta-sheet core; iv) feasible conjugation with various biological targets. To exemplify, oxazole yellow and its derivatives are virtually non-fluorescent in buffer solution but form stable intercalating complexes with double-stranded DNA, enhancing their fluorescence more than 1000 times [1-6]. Besides, a homodimeric derivative of oxazole yellow, YOYO-1, appeared to be especially useful for the detection of amyloid fibrils exhibiting about 200-fold emission enhancement upon binding to the Aβ(1-42) amyloid aggregates and a characteristic absorption shift originated from a self-stacking to non-stacking transition in its homodimer [14]. Notably, Cavuslar and Unal demonstrated a possibility of utilization of YO derivatives as fluorescent hybrid nanomaterials due to their light-up behavior when they interact with carbon nanotubes and DNA wrapped carbon nanotubes [10].

In the present study, the fluorescence spectroscopy and molecular docking techniques were used to explore the interaction of the novel monomethine cyanine oxazole yellow derivatives (Figure 1) with proteins in the native and amyloid states. To this end, the fluorescence spectral properties of YO-dyes were studied in the presence of native (non-fibrilar) and fibrilar insulin or lysozyme. More specifically, our aim was two-fold: i) to perform comparative analysis of

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the spectral behaviour and binding parameters of the cyanine dyes in the non-fibrilar and fibrilar proteins; ii) to determine potential binding sites for the novel dyes in the nonaggregated and fibrilar insulin and lysozyme.

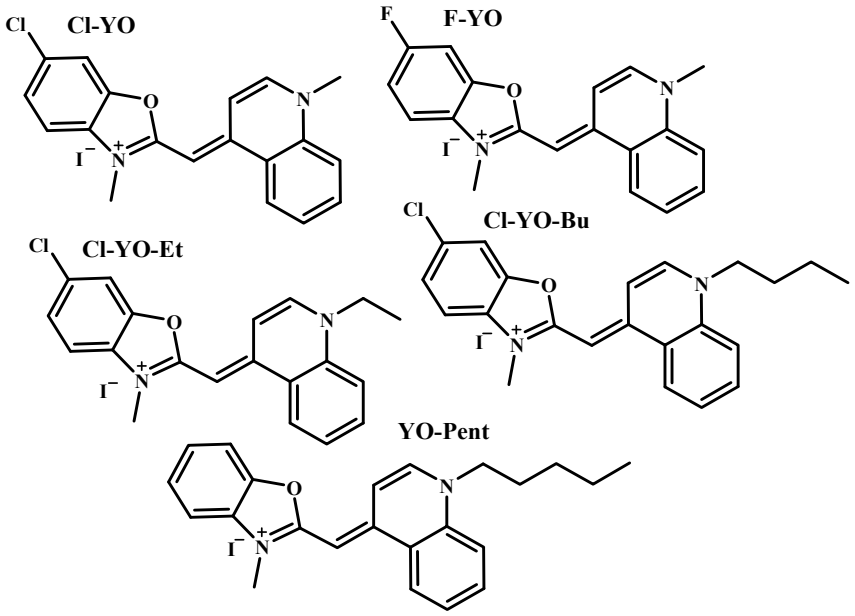

Figure 1. Structural formulas of monomethine cyanine dyes

EXPERIMENTAL SECTION

Materials

Bovine insulin (Ins), egg yolk lysozyme (Lz) and thioflavin T (ThT) were purchased from Sigma, USA. The monomethine cyanine dyes were kindly provided by Prof. Todor Deligeorgiev (Faculty of Chemistry, University of Sofia, Bulgaria). All other reagents were of analytical grade and used without the further purification. The structural formulas of the employed fluorescent dyes are shown in Fig. 1.

Preparation of working solutions

Stock solutions of the monomethine cyanines were prepared immediately before the fluorescence measurements by dissolving the dyes in dimethyl sulfoxide. The ThT stock solution was prepared in 5 mM sodium phosphate buffer, pH 7.4. The concentrations of cyanine dyes and ThT were determined spectrophotometrically using their molar absorptivities 72600 M^{-1} cm^{-1}, 60300 M^{-1} cm^{-1}, 64000 M^{-1} cm^{-1}, 90500 M^{-1} cm^{-1}, 90400 M^{-1} cm^{-1} and 36000 $M^{-1}cm^{-1}$ for Cl-YO, F-YO, Cl-YO-Et, Cl-YO-Bu, YO-Pent and ThT, respectively [12]. Working solutions of monomethines were prepared by dilution of the dye stock solutions in the sodium phosphate buffer, pH 7.4.

The insulin and lysozyme stock solutions (10 mg/ml) were prepared by dissolving the protein in 10 mM glycine buffer (pH 2.0). These solutions were used as references for non-aggregated proteins. Hereafter, the non-fibrillar insulin and lysozyme forms are designated as InsN and LzN, respectively. To prepare the insulin amyloid fibrils, this solution was subjected to constant agitation on the orbital shaker at 37 °C. Amyloid fibrils of lysozyme were obtained by the protein incubation in 10 mM glycine buffer at pH 2 and 60 °C for 14 days. The nature of the protein aggregates was confirmed by ThT assay and the transmission electron microscopy (data not shown). Hereafter, the fibrillar insulin and lysozyme are designated as InsF and LzF, respectively. The working solutions of proteins were prepared by dissolving a stock solution of the non-fibrillar or fibrillar insulin or lysozyme in 5 mM sodium phosphate buffer (pH 7.4).

Fluorescence measurements

Steady-state fluorescence spectra were recorded with LS-55 spectrofluorimeter (Perkin Elmer, UK) at 20 C using 10 mm path-length quartz cuvettes. Emission spectra were carried out in 5 mM sodium phosphate buffer (pH 7.4) and in the presence of non-fibrillar or fibrillar proteins with excitation wavelengths of 460 nm. The excitation and emission slit widths were set at 10 nm.

Binding model

Quantitative characteristics of the dye-protein binding were determined in terms of the Langmuir adsorption model by analyzing protein-induced changes in the probe fluorescence intensity at the wavelengths, corresponding to

emission maxima for each dye [16]. Briefly, approximation of the experimental dependencies ΔI (fluorescence intensity increase) on C_P (total protein concentration) by Eq. 1 allowed us to determine the dye-protein binding parameters – association constant (K_a), binding stoichiometry (n) and molar fluorescence (α), characterizing the difference between molar fluorescence of the bound and free dye:

$$\Delta I = 0.5\alpha\left[Z_0 + nC_P + 1/K_a - \sqrt{(Z_0 + nC_P + 1/K_a)^2 - 4nC_P Z_0}\right] \quad (1)$$

where Z_0 is the total probe concentration.

Molecular docking studies

To define the most energetically favorable binding sites for the examined dyes on the non-fibrillar and firbrillar proteins, the molecular docking studies were performed using the AutoDock (version 4.2) incorporated in the PyRx software (version 0.8) [17]. The chromophore structures were built in MarvinSketch (version 18.10.0) and optimized in Avogadro (version 1.1.0) [18,19]. Crystal structures of hen egg white lysozyme (PDB ID: 3A8Z) and bovine insulin (PDB ID: 2ZP6) were taken from the Protein Data Bank. Lysozyme fibril was built from the K-peptide, GILQINSRW (residues 54–62 of the wild-type protein), using CreateFibril tool as described previously [20]. The model of the human insulin fibril was obtained from http://people.mbi.ucla.edu/sawaya/jmol/fibrilmodels/. The selected docking poses were visualized with the UCSF Chimera software (version 1.14) [21].

RESULTS AND DISCUSSION

To explore the interaction of the novel monomethine cyanine dye with proteins in globular and amyloid states, at the first step of the study the fluorescence spectral properties of these dyes were studied in the unbound state and in the presence of native and fibrillar insulin and lysozyme. Figure 2 represents typical fluorescence spectra recorded upon titration of cyanine dyes with proteins.

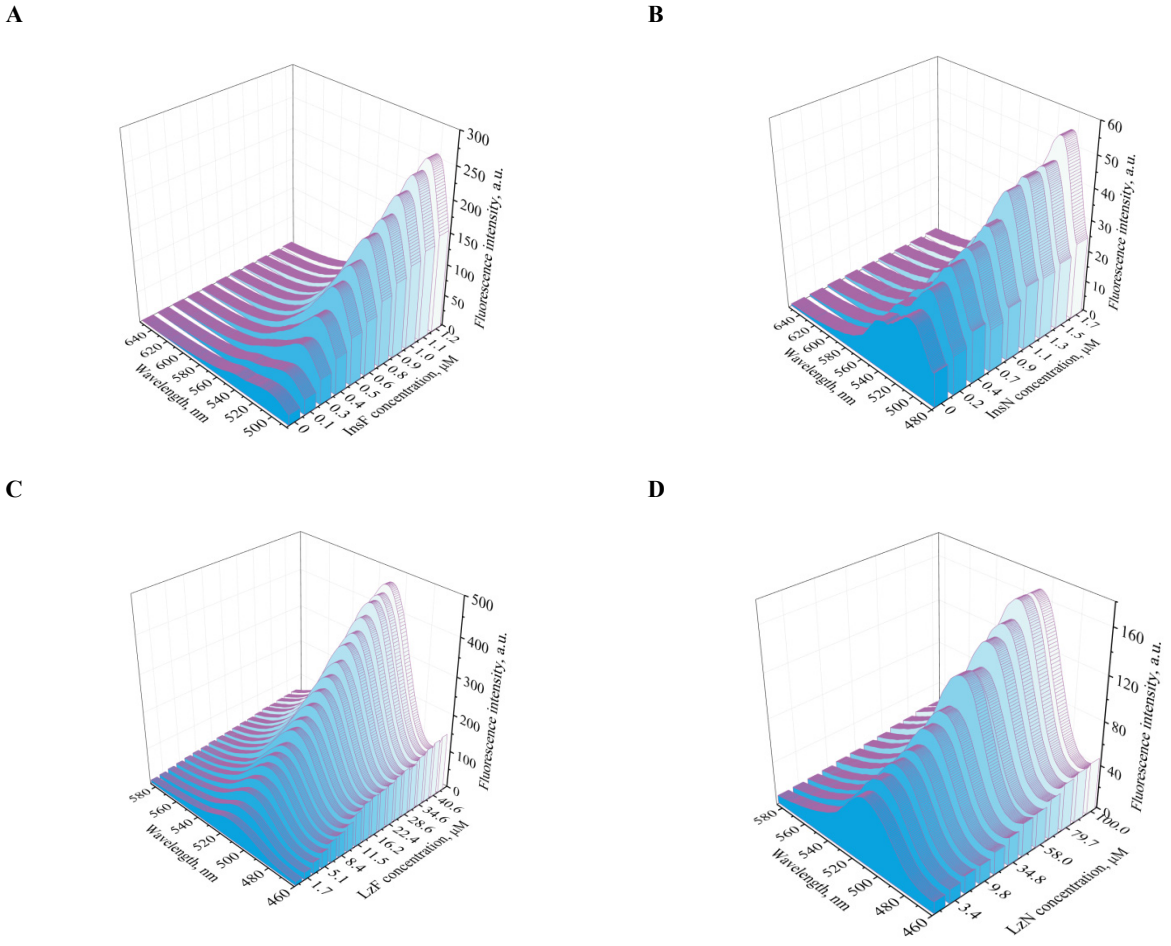

Figure 2. Typical emission spectra of Cl-YO-Bu recorded upon the dye fluorimetric titration with InsF (A), InsN(B), LzF (C) and LzN (D). Dye concentrations were 0.1 μM (A, B) and 0.4 μM (C, D), respectively.

The spectral characteristics of cyanine dyes in the presence of the non-fibrillar and fibrillar proteins were evaluated and summarized in Table 1. As seen in Table 1, all examined cyanine dyes exhibit a weak fluorescence in the

buffer solution with the emission maxima centered in the range 510-513 nm depending on the dye chemical structure. The addition of the non-fibrillar insulin resulted in a ~ 10 nm hypsochromic shift of the emission maxima of monomethines, along with a slight fluorescence intensity increase. Elevating concentrations of the native lysozyme resulted in a more pronounced change of the cyanine emission coupled with a slight (3-5 nm) shift of the emission maxima to the lower wavelengths. Such behavior may be a consequence of a higher affinity of these dyes for lysozyme protein monomers in comparison with insulin. As shown in Table 1, the spectral response of the cyanines to the fibrillar proteins lies in a strong increase of the dye fluorescence (I_f) as opposed to that in buffer (I_0) and in the presence of nonfibrillar protein (I_n), with a magnitude of the fluorescence intensity increase being more pronounced in the presence of the insulin amyloid fibrils. The fluorescence maxima of the monomethines are shifted by approximately 3-6 nm (depending on the dye chemical structure) towards shorter wavelengths compared to those observed in the non-aggregated proteins. These effects can be explained by a fluorophore transfer into a less polar and motionally restricted protein environment. Moreover, the emission spectra for all monomethine dyes bound to the lysozyme fibrils were red shifted for 5-9 nm (depending on dye structure) in comparison with those in the presence of the fibrillar insulin. The above finding most probably indicates that the cyanine dyes bind to more polar binding sites in the lysozyme amyloid fibrils than that on the insulin fibrils. Notably, the sensitivity of the dye spectral properties to the changes in the protein environment was previously reported for Michler's hydrol blue and Nile red [22,23].

Table 1. Spectral characteristics of the monomethine cyanine dyes in buffer and in the presence of native and fibrillar proteins

	Free dye		**InsN**		**InsF**		**ADF**	**LzN**		**LzF**		**ADF**
	λ_{em}	I_0	λ_{em}	I_n	λ_{em}	I_f		λ_{em}	I_n	λ_{em}	I_f	
Cl-YO	513	34.3	502	69.2	501	300.6	6.7	509	142.7	506	366.4	3.71
Cl-YO-Bu	511	31.9	506	47.4	501	258.1	6.3	505	167.1	507	473.9	5.5
Cl-YO-Et	513	34.4	502	34.7	499	220.9	5.4	509	105.9	507	215.2	2.2
F-YO	511	19.6	501	27.8	499	198.1	8.4	511	64.1	508	114.8	2.5
YO-Pent	510	53.7	502	63.0	500	354.3	5.3	508	94.1	506	327.6	3.47

λ_{em} (nm)– the lowest energy maximum of the excitation spectra; I_0 , I_n , and I_f – fluorescence intensity of the dyes in buffer and in the presence of nonfibrillar and fibrillar proteins, respectively. Nonfibrillar and fibrillar insulin concentrations were 1.2 μM. Native and fibrilar lysozyme concentrations were 44 μM.

To determine the specificity of the examined cyanines to amyloid fibrils, the fluorescence spectral data were processed to the amyloid detection factor (ADF) characterizing the ability of a dye to selectively detect the fibrillar state over its native structure relative to the background fluorescence of the dye in buffer [24,25]:

$$ADF = \frac{I_f - I_n}{I_0} \tag{2}$$

It appeared that all dyes under study in the presence of the fibrillar insulin and lysozyme are characterized by the positive ADF values testifying to the higher sensitivity of monomethines to the fibrillar protein aggregates compared to the non-aggregated state. The ADF values for the fibrilar insulin were found to rise in the order YO-Pent → Cl-YO-Et → Cl-YO-Bu → Cl-YO → F-YO, indicating the increase of insulin amyloid selectivity from Yo-Pent to F-Yo dyes. It is worth noting that the long aliphatic substitution on the nitrogen atom of the benzazole chromophore reduces the amyloid specificity of the monomethines (Cl-YO-Et, Cl-YO-Bu, YO-Pent compared to Cl-YO, F-YO). Thus, the steric hindrances may result in the decrement of the number of available protein binding sites for Cl-YO-Et, Cl-YO-Bu and YO-Pent, which possess the bulky alkyl substitutions. Besides, in the presence of lysozyme fibrils the amyloid specificity was found to decrease in the following row Cl-YO-Bu → Cl-YO → YO-Pent → F-YO → Cl-YO-Et. Remarkably, ADF values appeared to be significantly higher in the presence of insulin amyloid fibrils in comparison with lysozyme, suggesting a sensitivity of the examined monomethine cyanines to the fibril morphology.

Next, to derive the quantitative parameters of the dye-protein binding, the experimental dependencies of the measured fluorescence intensity increase (ΔI) *vs* protein concentration (C_P) (Figure 3) were approximated by the Langmuir adsorption model Eq. 1. The quantitative analysis of the dependencies $\Delta I(C_P)$ allowed us to estimate the parameters of the monomethine complexation with native and fibrillar proteins − association constant (K_a), binding stoichiometry (n) and the difference between the molar fluorescence of the bound and free dye (α) (Table 2).

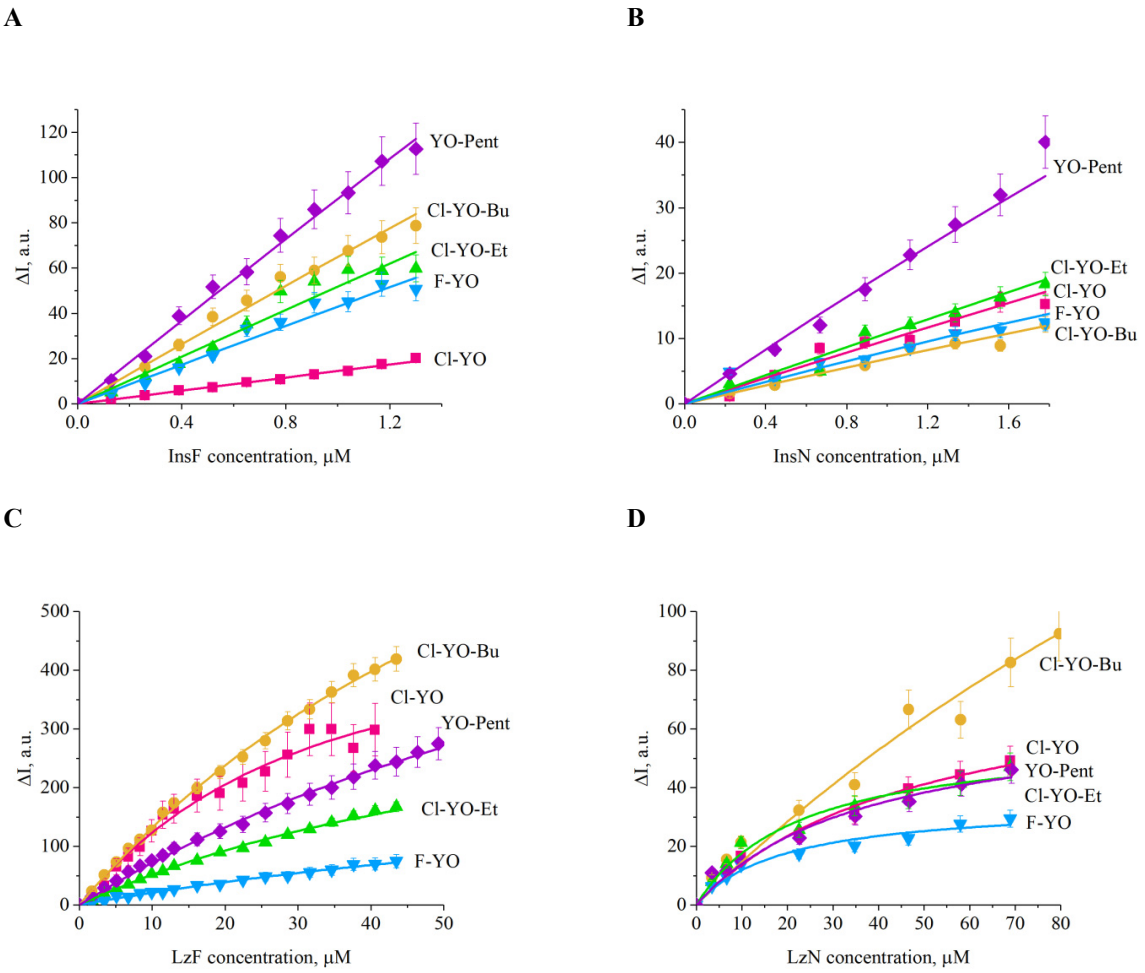

Figure 3. The isotherms of the cyanine dyes binding to InsF (A), InsN(B), LzF (C) and LzN (D). Dye concentrations were 0.1 μM (A, B) and 0.4 μM (C, D), respectively.

The results presented in Table 2 indicate that monomethine dyes possess relatively high association constant in the presence of nonfibrillar and fibrillar proteins. Besides, the K_a values for the examined dyes were ~ 2–30 times higher for the native proteins in comparison with the fibrillar insulin and lysozyme, whereas the values of the molar fluorescence (α) were higher in the presence of the amyloid fibrils. All above findings show that despite the positive ADF values, the dyes under study readily associate also with the monomeric (native) proteins.

Table 2. Binding characteristics of cyanine dyes in the presence of the non-aggregated and fibrillar proteins

Dye	InsN			InsF			LzN			LzF		
	$Ka \cdot 10^3$, μM^{-1}	n	$\alpha \cdot 10^{-3}$, μM^{-1}	$Ka \cdot 10^3$, μM^{-1}	n	$\alpha \cdot 10^{-4}$, μM^{-1}	$Ka \cdot 10^3$, μM^{-1}	n	α, μM^{-1}	$Ka \cdot 10^3$, μM^{-1}	n	$\alpha \cdot 10^{-3}$, μM^{-1}
Cl-YO	32.36	0.68	4.54	1.92	0.29	25.52	44.92	1.31	85.54	10.77	0.99	0.59
Cl-YO-Bu	57.69	0.73	2.54	29.15	0.62	5.40	35.88	1.05	175.35	15.8	1.0	2.15
Cl-YO-Et	19.66	0.98	5.57	10.33	0.59	8.32	58.26	1.02	102.46	5.32	0.98	2.05
F-YO	72.25	1.00	1.07	14.85	0.57	4.50	77.7	1.01	73.04	63.03	1.03	0.17
YO-Pent	47.39	1.02	4.39	34.28	0.62	4.32	107.42	0.79	83.81	21.85	1.01	1.25

It should also be noted that despite the observed positive ADF values of the cyanine dyes under study, their amyloid specificity is significantly lower in comparison with other amyloid markers [24-28]. In particular the estimated ADF values for the previously reported trimethine cyanine dyes [24] and phenyleneethynylene-based dyes [25] were about twice as large as those calculated in the present work. Moreover, the enhancement of the fluorescence intensity (ratio I_f / I_0) in the presence of amyloid fibrils for the all dyes under investigation didn't exceed 10, while for some

cyanine dyes [24, 26], benzanthrone derivatives [27] and β-ketoenoles [28] binding preferences were significantly higher (values of I_f / I_0 ratios were more than 50). Specifically, the binding of a homodimeric derivative of oxazole yellow YOYO to amyloid fibrils was found to be accompanied by a 200-fold emission enhancement [14]. Therefore, the following correlation between the dye structural properties and their amyloid sensing potential can be suggested:

1) The introduction of chloro- (Cl-YO-Et, Cl-YO-Bu, Cl-YO) and fluorine-substitutients (F-YO) to the oxazole yellow derivatives had a negative impact on the dye amyloid specificity. It should be noted in this respect that in contrast, the chloro-substituent at YOYO structure resulted in a significant increase of the dye affinity for DNA and RNA relative to the parent compounds YOYO [4].
2) The long aliphatic substituents on the nitrogen atom of the benzazole chromophore can be responsible for a reduction of the monomethine binding to amyloid fibrils (Cl-YO-Et, Cl-YO-Bu, YO-Pent compared to Cl-YO, F-YO)

To identify the monomethine-protein binding sites, as well as the nature of the interactions involved in the dye–protein complexation in the non-fibrillized proteins and amyloid fibrils, we used the molecular docking technique. The possible binding sites of the dyes under study within the native (Figure 4 panels A, B) and fibrillar (Figure 4, panel E) insulin were identified using the AutoDock tools. All monomethines under study tend to form the most stable complexes with the B-chain residues (Val 17, Leu17, Ala 14, Phe1, Gln 4 and Leu 6) and the residues Leu 13, Tyr 14, Glu 17 of the A-chain of insulin. The protein-ligand interaction profiler PLIP (https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index) was used to further characterize the nature of the dye-protein interactions [29]. The binding of the investigated cyanines is governed predominantly by hydrophobic interactions between the dye molecule and the insulin residues Val 17, Leu17, Ala 14, Gln 4 and Leu 6, Leu 13, Tyr 14, Glu 17 (gray dashed lines on the panels C and D, Figure 4). Moreover, it was found that all examined dyes tend to form π-stacking contacts between benzene ring and Phe1 residue of the B-chain of the non-fibrilar insulin (green dashed line on the panel D, Figure 1). The docking results with the fibrilar insulin showed that the wet surface groove Gln15_Glu17 provides the most energetically favorable binding site for all examined monomethine dyes with the binding affinity for the docked poses equal to -6.05 kcal/mol (Cl-YO), -6,09 kcal/mol (Cl-YO-Bu), -5.72 kcal/mol (F-YO), -5,95 kcal/mol (Cl-YO-Et) and -5,48 kcal/mol (YO-pent).

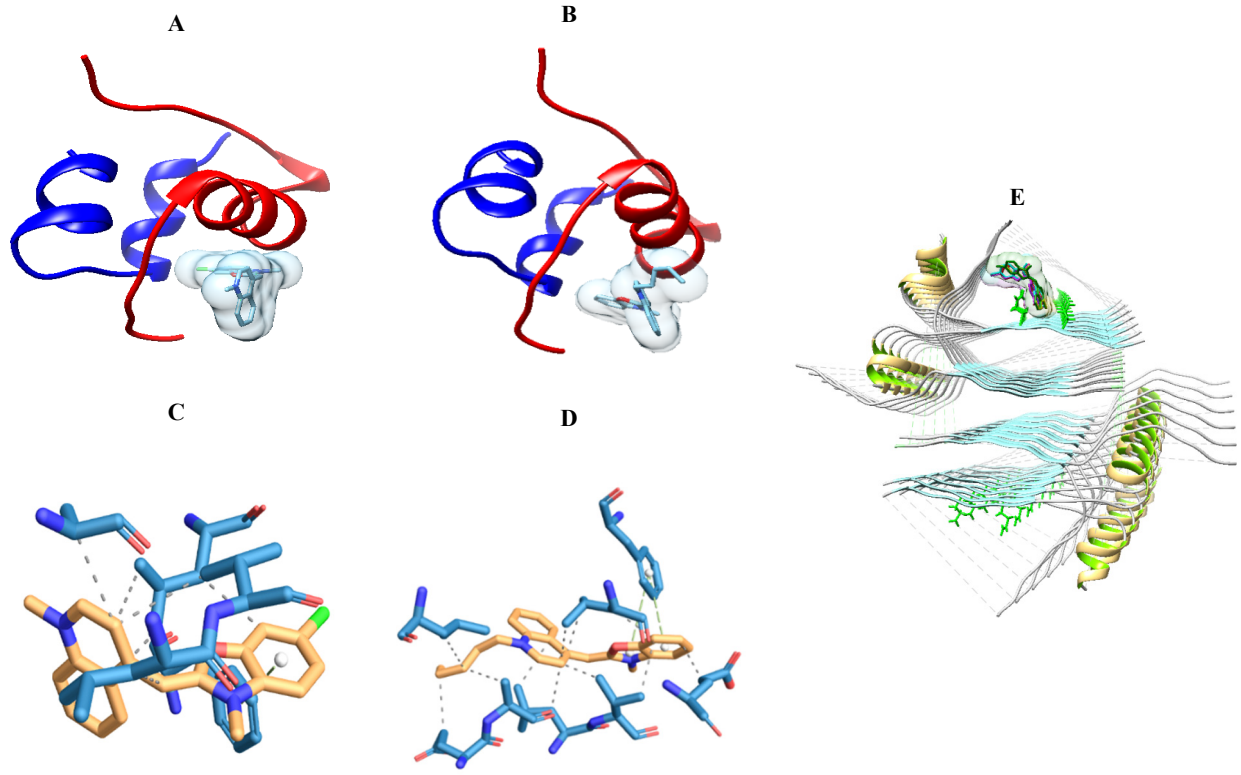

Figure 4. Schematic representation of the energetically most favourable dye complexes with the non-fibrillized and fibrillar insulin, obtained using the AutoDock (panels A, B and E) and the protein–ligand interaction profiler PLIP (panels C and D). The panels A and C represent Cl-YO interactions with non-aggregated insulin. Shown in the panels B and D is association of YO-Pent with the B-chain and the A-chain residues of the non-fibrillized protein. The panel E represents the binding of Oxazole yellow derivatives (Cl-YO – green, Cl-YO-Bu – purple,Cl-YO-Et – blue, F-YO – yellow,YO-Pent – red) to the fibrillar insulin.

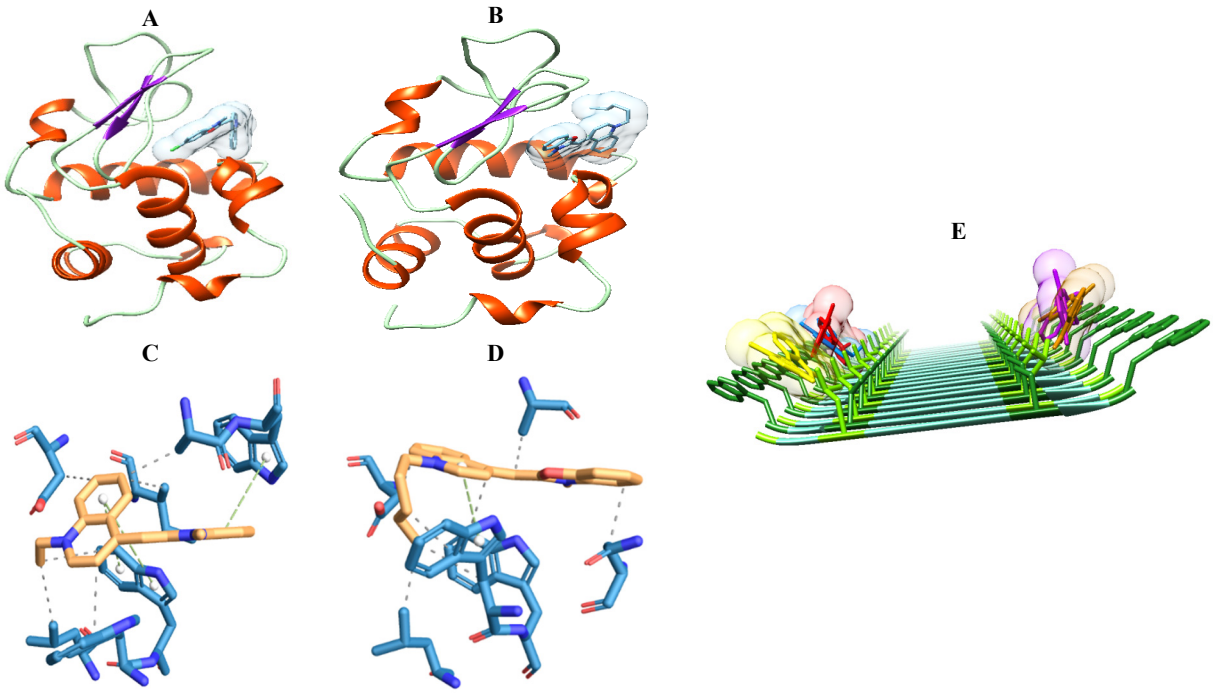

Figure 5. Schematic representation of the energetically most favourable dye complexes with the native and fibrillar lysozyme, obtained using the AutoDock (panels A, B and E) and the protein–ligand interaction profiler PLIP (panels C and D). The panels A and C represent Cl-YO-Et interactions with native lysozyme. Shown in the panels B and D is association of YO-Pent with the deep cleft of native lysozyme. The panel E represents the binding of monomethines (Cl-YO – brown, Cl-YO-Bu – purple, Cl-YO-Et – blue, F-YO – yellow, YO-Pent – red) to the groove G2-L4/S8-W10 of the lysozyme fibril.

The novel monomethine dyes interact with the deep cleft of native lysozyme lined with both hydrophobic (Ile98, Ile 58), polar (Thr108, Thr 62 and Thr 63 residues) and negatively (Asp101, Asp 107) charged residues (Figure 5, panels A-D). Similarly to the non-fibrillar insulin, the results obtained using PLIP serve indicate that the dye-lysozyme association is predominantly driven by the hydrophobic dye–protein interactions (dashed grey lines on the panel C and D, Figure 5). Moreover, the docking studies predict that there is a π-stacking interaction between the benzene ring of the dye with Trp 62. The role of the π-stacking interactions in the dye-lysozyme complexation was previously reported also for Azo dyes [30]. Figure 5 D represents that monometines under study are prone to form the energetically most favourable dye complexes with the groove G2-L4/S8-W10 of the lysozyme fibril. Similarly, classical amyloid marker Thioflavin T preferentially interacted with the grooves, containing aromatic residues [31].

CONCLUSIONS

To summarize, the present study was focused on the investigation of the interactions between the novel monomethine cynine dyes and proteins in the non-aggregated and fibrillar states. Using the fluorescence spectroscopy technique it was found, that the oxazole yellow derivatives are readily associate with both non-fibrillar and fibrillar insulin or lysozyme with the magnitude of the dye-protein complexation being higher for the fibrillar proteins. The comparison of the fluorescence responses and the binding parameters in the presence of the fibrillar insulin and lysozyme led us to hypothesize, that novel monomethine dyes are sensitive to the fibril morphology. It was found that the introduction of chloro- and fluorine-substitutients to the oxazole yellow derivatives, as well as the long aliphatic substituents on the nitrogen atom of the benzazole chromophore of YO-dyes had a negative impact on the dye amyloid specificity. Based on the molecular docking modeling, it was found that the dye–protein hydrophobic and π-stacking interactions are supposed to have the predominant influence on the association of monomethines with proteins in the non-aggregated and fibrilar states. Overall, the obtained results can be useful for the development and optimization of fluorescent probes for amyloid fibril detection.

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ВЗАЄМОДІЯ НОВИХ МОНОМЕТИНОВИХ ЦІАНІНОВИХ БАРВНИКІВ З БІЛКАМИ У НАТИВНОМУ ТА АМІЛОЇДНОМУ СТАНАХ

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За допомогою методів флуоресцентної спектроскопії та молекулярного докінгу проведено дослідження молекулярних механізмів взаємодії між новими монометиновими ціаніновими зондами і нефібрилярними та фібрилярними білками. З цією метою, були досліджені флуоресцентні спектральні властивості зондів в буфері та в присутності інсуліну та лізоциму в нативному та амілоїдному станах. Встановлено, що асоціація монометинів з нефібрилярними та фібрилярними білками

супроводжується значним зростанням інтенсивності флуоресценції барвників, яке було більш вираженим у присутності агрегованого інсуліну та лізоциму. Шляхом апроксимації експериментальних залежностей збільшення інтенсивності флуоресценції флуорофору від концентрації білка моделлю Ленгмюра було отримано кількісну інформацію щодо зв'язування зондів з білками. Аналіз спектральних властивостей і отриманих параметрів зв'язування монометинів з фібрилярним інсуліном та лізоцимом показав, що хлор- і фтор-замісники в структурі похідних оксазолового жовтого, а також довгі аліфатичні групи на атомі азоту бензазолового фрагменту YO-барвників негативно впливають на амілоїдну специфічність барвників. З використанням молекулярного докінгу показано, що монометини мають тенденцію утворювати найбільш стабільні комплекси із залишками Val 17, Leu17, Ala 14, Phe1, Gln 4 і Leu 6 B-ланцюга і залишками Leu 13, Tyr 14, Glu 17 A-ланцюга нефібрилярного інсуліну та з гідрофобними (Ile98, Ile 58), полярними (Thr108, Thr 62, Thr 63) і негативно зарядженими (Asp101, Asp 107) амінокислотними залишками нативного лізоциму. Показано, що жолобки фібрил, що утворені амінокислотними залишками Gln15_Glu17 в фібрилярному інсуліні та G2-L4/S8-W10 в фібрилах лізоциму є найбільш енергетично вигідними сайтами зв’язування для монометинових зондів.

Ключові слова: Монометинові ціанінові зонди, інсулін, лізоцим, молекулярний докінг